

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q97193

Si Young CHO, et al.

Appln. No.: 10/599,290

Group Art Unit: 4131

Confirmation No.: 6567

Examiner: Bahar Schmidtman

Filed: March 25, 2008

For: COMPOSITION CONTAINING GINSENOSE F1 AND EGCG FOR PREVENTING
SKIN DAMAGE

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Ms. Si-Young CHO, hereby declare and state:

THAT I am a citizen of Republic of Korea;

THAT I have received the degree of doctor of philosophy in Molecular biology from
Korea University;

THAT I have been employed by Amorepacific Corporation since 2002, where I hold a
position as principal scientist, with responsibility for research of anti-aging effects of natural
compounds;

THAT I am one of the inventors of the above-identified application; and

THAT I reviewed the Office Action mailed July 21, 2009, in which claims 1-5 and 8-10
are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al. (J. of Invest. Dermat.)
and Ahn et al. (KR 10-2003-0075492, abstract), in which the Ahn reference (KR 10-2003-

0075492) is cited as teaching a cosmetic composition comprising epigallocatechin-3-gallate (EGCG) that inhibits aging of the skin.

In order to show unexpectedly superior synergic effects of the combination of ginsenoside F1 and EGCG over other combinations of known antiapoptotic agents, the following tests were performed by me or under my direction and supervision.

In Example 1 in the specification of the instant application, Applicants showed that the combination of 2 μ M ginsenoside F1 + 10 μ M EGCG showed about 2-fold inhibitory effects on UV-caused apoptotic cell death compared to untreated control groups, while 2 μ M ginsenoside F1 alone or 10 μ M EGCG alone did not show any anti-apoptotic effects compared to the untreated control groups. In order to show the synergic effects were not predicted or obvious, Applicant performed further experiments on the combinations of ginsenoside F1 + Quercetin, EGCG + Quercetin, and Quercetin alone (in various concentrations).

Experiment Method

Anti-apoptotic effects of combined treatment with ginsenoside F1, Quercetin and EGCG in HaCaT cells were tested as follows:

[Step 1] Cell line and cell culture

Cell lines used in this experiment and the culture thereof were the same as used in Step 1 of Example 1.

[Step 2] Inhibition of UV-induced apoptosis in HaCaT cells by a combined treatment with ginsenoside F1 + Quercetin, or EGCG + Quercetin

Quercetin was reported as anti-apoptotic agent. E.g., Kim and Jang, "Protective mechanism of quercetin and rutin using glutathione metabolism on HO-induced oxidative stress in HepG2 cells," Ann. N. Y. Acad. Sci. 2009 Aug., 1171:530-7. A copy of Kim reference is submitted under a separate letter.

Cell lines cultured in Step 1 were treated with trypsin to give a single-cell suspension and seeded into a 6-well microplate at 2×10^5 cells per well, then cultured for 24 hours. Subsequently, the culture medium was refreshed with serum-free DMEM and cells were cultured for another 24 hours. The microplate was then treated with 5 μ M Quercetin; 25 μ M Quercetin; a combination of 2 μ M ginsenoside F1 + 5 μ M Quercetin; 5 μ M Quercetin + 10 μ M EGCG. For reference, ginsenoside F1 was dissolved in 100% ethanol at a 1/1000-fold concentration to the medium and EGCG (Sigma) and Quercetin (Sigma) were dissolved in dimethyl sulfoxide (DMSO) at a 1/1000-fold concentration to the medium, and then added at the required amount to the culture mediums.

After 24-hour treatment of each test sample, each microplate was washed with phosphate buffered saline (PBS) and exposed to 60 mJ/cm² of UVB in the presence of PBS. PBS was then removed and the culture medium was refreshed with a medium containing each compound at the corresponding concentration. As a control, untreated cells were cultured in the same way.

24 hours after UV irradiation, to the microplates treated with each compound or untreated was added 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) solution, and then the cells were cultured at 37°C for 4 hours. DMSO was added and dissolved completely. The optical density (OD) of formazan formed at 540 nm was measured using an

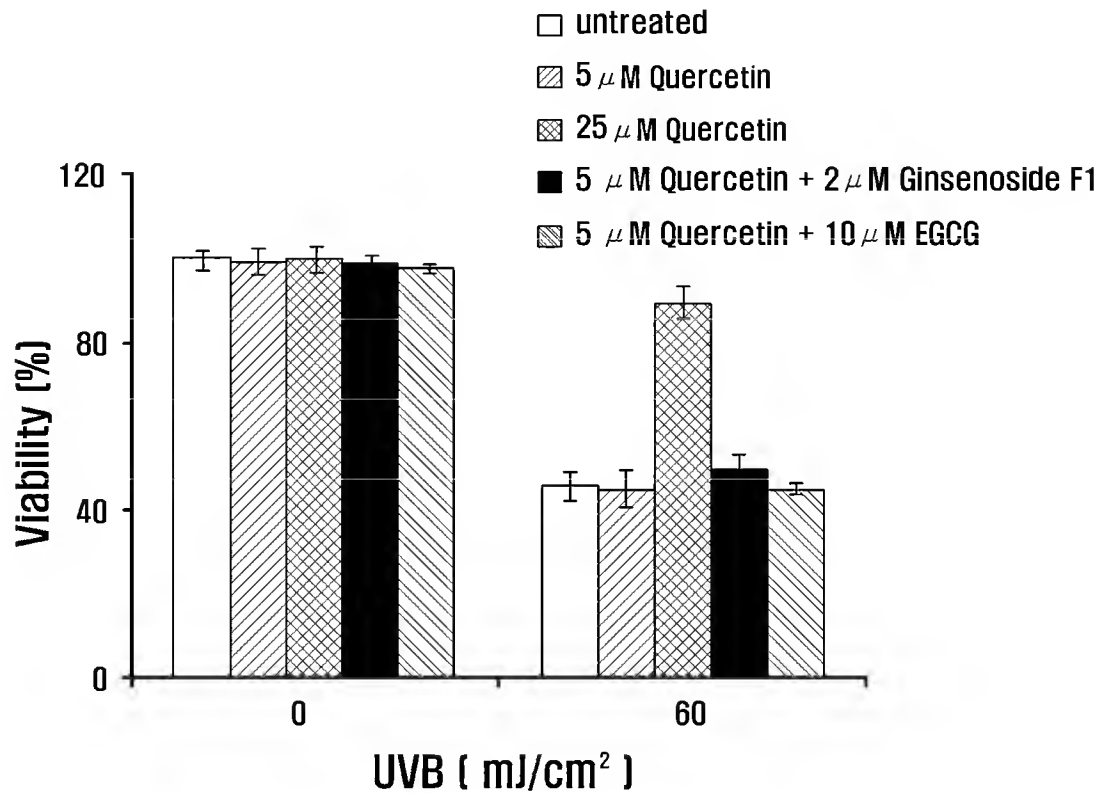
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ELISA reader (Thermo Max, Molecular Devices Co.). Cell viability in each test group was evaluated as a relative value, considering OD of untreated control cells as 100%. The results are shown in the Figure below.

As shown in the Figure, the treatment with 5 μ M Quercetin alone showed no difference in UV-caused apoptotic cell population compared with untreated control cells, although treatment of 25 μ M Quercetin inhibited UV-caused apoptotic cell death, as reported in a previous study (e.g., Kim reference (2009)). However, **combined treatments with 2 μ M ginsenoside F1 + 5 μ M Quercetin or 5 μ M Quercetin + 10 μ M EGCG did not inhibit UV-caused apoptotic cell death.** That is, **the combinations of ginsenoside F1 or EGCG with other known anti-apoptotic agent (Quercetin) do not show any synergic effects.**

Figure




Conclusion

The results of the above experiments and the results presented in the specification of the instant application clearly show that the synergic effects of the combined use of ginsenoside F1 and EGCG at much lower concentrations than the effective concentrations of respective individual agents on inhibiting UV-caused apoptotic cell deaths are not predictable or obvious.

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I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: December 16, 2009



Ms. Si-Young CHO